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A Practical Methodology to Measure Unbiased Gas Chromatographic Retention Factor vs. Temperature Relationships

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Abstract

Compound identification continues to be a major challenge. Gas chromatography-mass spectrometry (GC-MS) is a primary tool used for this purpose, but the GC retention information it provides is underutilized because existing retention databases are experimentally restrictive and unreliable. A methodology called “retention projection” has the potential to overcome these limitations, but it requires the retention factor (k) vs. T relationship of a compound to calculate its retention time. Direct methods of measuring k vs. T relationships from a series of isothermal runs are tedious and time-consuming. Instead, a series of temperature programs can be used to quickly measure the k vs. T relationships, but they are generally not as accurate when measured this way because they are strongly biased by non-ideal behavior of the GC system in each of the runs. In this work, we overcome that problem by using the retention times of 25 n -alkanes to back-calculate the effective temperature profile and hold-up time vs. T profiles produced in each of six temperature programs. When the profiles were measured this way and taken into account, the k vs. T relationships measured from each of two different GC-MS instruments were nearly as accurate as the ones measured isothermally, showing less than 2-fold more error. Furthermore, temperature-programmed retention times calculated in five other labs from the new k vs. T relationships had the same distribution of error as when they were calculated from k vs. T relationships measured isothermally. Free software was developed to make the methodology easy to use. The new methodology potentially provides a relatively fast and easy way to measure unbiased k vs. T relationships.

Keywords

Retention projection; Retention prediction; Gas Chromatography-Mass Spectrometry; Retention library; Retention database

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1. Introduction

The identification of small molecules continues to be a major bottleneck in the analysis of complex mixtures. Typically only a small fraction of the compounds in a sample can be identified with high confidence, requiring meticulous work by a skilled individual. Of the analytical tools available, gas chromatography-mass spectrometry (GC-MS) is one of the primary tools used for this purpose. It provides two complementary pieces of information that can be used for identification: mass spectra and chromatographic retention information. To identify a compound by GC-MS, one runs samples of potential chemical identities and eliminates ones that have significantly different mass spectra and retention times, ideally leaving only one potential identity remaining.

However, it is often impractical to obtain a sample of every potential chemical identity, so we must rely on shared databases of mass spectral and retention information to make identifications. Though mass spectral databases have found wide use for compound identification, shared GC retention databases have found relatively limited use despite their potential value for compound identification. There are a number of reasons for this. First, in order to reproduce the retention data, one is limited to using precisely the same experimental conditions that were used to build the database [1–3] (or to one of a narrow range of translated methods [4–6]). But even then, it is almost impossible to strictly reproduce the experimental conditions used to develop the database because the retention data is biased by non-ideal behavior of the GC system used to measure them (by “non-ideal” GC system behavior, we mean behavior that deviates from that of an ideal GC system: temperature calibration error, flow rate error, imprecise column dimensions, etc.) [7]. Because of this, it is unclear how much error one should expect if the shared retention data is used across different systems, making it difficult to use shared retention information to reject a potential identity on solid statistical grounds.

Currently, the most common way to share retention data in temperature-programmed GC runs is as linear retention indices (LRIs) [8]. LRIs describe the position a compound elutes between a pair of bracketing standards. Since they are calculated relative to the retention times of two other compounds subjected to the same experimental conditions, the idea is that they should be less sensitive to the small variations in the experimental conditions used to measure them. They are indeed less sensitive to them than absolute retention times, but they are still strongly affected by them. In fact, LRIs are affected by a change in almost any experimental condition: the temperature program, the flow rate/inlet pressure, the outlet pressure, the column length, the inner diameter, and the stationary phase film thickness. Even relatively small non-idealities in those experimental parameters have been found to cause significant shifts [7]. Retention time locking can be used in combination with linear retention indexing to improve its reproducibility across GC systems by calibrating out differences in hold-up time between the two GC systems [4]. However, it provides no way to account for non-idealities in the temperature program and a user is still limited to using precisely the same experimental conditions as were originally used to measure the data (or to one of a narrow range of translated methods).

A far less restrictive way to share GC retention information is to compile a database of isothermal retention factors (k) as a function of temperature. Then, temperature-programmed retention times are calculated by considering the temperature program as a series of very short isothermal steps as in eq. 1 (which is analogous to the integral, but can be solved with more complicated, non-linear t_M vs. T , T vs. time, and $\ln k$ vs. T relationships) [9–14]:

$$\sum_{i=1}^n \frac{\delta t}{t_{M,T}(k_T+1)} \geq 1 \quad (1)$$

where $t_{M,T}$ is the hold-up time k_T is the retention factor at the T of the step, and n is the smallest integer that makes the inequality true. In each step, the fraction of the column traveled by the compound is calculated based on its k at the T of that step and the t_M at that T . Its retention time, t_R , is then calculated from the time required for the compound to travel the entire length of the column:

$$t_R = \sum_{i=1}^n \delta t \quad (2)$$

We call this approach “retention projection” because temperature-programmed retention times are “projected” from isothermal k vs. T relationships. (Stated another way, the static k vs. T relationships manifest themselves as different retention times when they are “projected” onto different experimental conditions.) The major advantage of this approach is that the k vs. T relationships can be used to calculate a compound’s retention time under a wide range of temperature programs, flow rates/inlet pressures, outlet pressures, and column dimensions. Only the stationary phase and the carrier gas must be fixed. Furthermore, when this approach is combined with a novel back-calculation algorithm to account for GC system non-idealities (see section 1.1), we have found retention projections to be robust and considerably more accurate than retention indexing when used across labs [7]. More importantly, the methodology was found to account for virtually all differences between labs and methods, making it possible to calculate the appropriate retention time tolerance window for each projected retention time with a known, absolute level of confidence.

Due to these and other benefits, we considered building a larger database of isothermal k vs. T relationships to make the retention projection methodology more broadly useful for compound identification. The most straightforward way to measure these k vs. T relationships is to directly measure k in a series of isothermal runs over a range of temperatures, however, this approach is not practical for large numbers of compounds. First, it takes a long time—if the retention of compounds in a mixture span a wide range, it is necessary to measure retention at 10 to 15 different temperatures to ensure collection of enough retention factors for both poorly-retained and well-retained compounds. Data collection at each temperature takes about 1.5 hours to allow the temperature to equilibrate, to make the hold-up time measurements, to run the sample mixture, and to clear out the column at high temperature to prepare it for the next run. Second, a high accuracy temperature probe and careful annotation of the true temperature for each measurement is required to avoid bias from temperature calibration error, which adds further complication and extra equipment.

Instead of directly measuring k vs. T relationships from a series of isothermal measurements, a faster approach involves running a set of temperature programs and using a compound's retention time in each run to solve for its k vs. T relationship [15–18]. The solution is found iteratively, by adjusting a k vs. T relationship until the projected retention times in each temperature program are as close as possible to the measured retention times. To constrain the possible solutions, an equation is used to describe the k vs. T relationships. The following thermodynamic relationship has been shown to fit these relationships with good precision [19–21,17]:

$$k = e^{A + \frac{B}{T} + C \ln(T)} \quad (3)$$

$$A = \frac{\Delta S(T_0) - \Delta C_p \ln(T_0) - \Delta C_p}{R} \quad (4)$$

$$B = - \frac{\Delta H(T_0) - \Delta C_p T_0}{R} \quad (5)$$

$$C = \frac{\Delta C_p}{R} \quad (6)$$

where T is the temperature, T_0 is a reference temperature (here we use 273.15 K), $\Delta H(T_0)$ and $\Delta S(T_0)$ are the changes in molar enthalpy and entropy for transfer of the analyte from the gaseous mobile phase into the stationary phase at the reference temperature, and ΔC_p is the change in its isobaric heat capacity for the transfer. Thus, with this equation, three parameters describe a compound's k vs. T relationship: $\Delta H(T_0)$, $\Delta S(T_0)$, and ΔC_p .

While this approach is relatively fast, it can introduce considerable bias into the measurement. In its simplest form, the assumption is usually made that both the temperature profile and the t_M vs. T profiles produced by the GC system are ideal, which is rarely the case, thereby introducing bias into the k vs. T relationships. McGinitie et al. recently reported a protocol to measure and account for some system non-idealities [18]. First, the column was rolled out and its precise length was measured. Then the column was rewound, installed, and the t_M was measured at three temperatures by injecting methane, which was then used to calculate the column's effective inner diameter. Then, the Grob test mixture was run under six different temperature programs and a custom MATLAB script was used to iteratively solve for the effective film thickness. Finally, sets of six temperature programs were run to iteratively solve for values of $\Delta H(T_0)$, $\Delta S(T_0)$, and ΔC_p of individual compounds using the above measurements of column length, effective inner diameter, and effective film thickness using another custom MATLAB script.

While the protocol described by McGinitie et al. made a strong attempt to account for bias resulting from the column, in our view, it is not a viable solution. First, the amount of effort and expertise required is substantial. A typical GC user is unlikely to use such a methodology to calibrate their system. The solution is also incomplete; it does not account for temperature inaccuracy and it assumes that the column inlet and outlet pressures are

ideal. Of course, they could be taken into account by careful measurement, but it would make the protocol even more time consuming and impractical.

1.1 Back-calculation of the effective GC system behavior

However, we recently described a new approach that makes it relatively easy to measure the unintentional errors in the temperature program and t_M vs. T relationship [22]. Briefly, a sample is spiked with a series of n -alkane standards and run in the desired temperature program for the analysis (currently, the DB5MS UI phase and He carrier gas must be used). The retention times of the n -alkanes are then entered into online software we developed (www.retentionprediction.org/gc). *The software uses the retention times of the n -alkanes to back-calculate the temperature and t_M vs. T profiles that were produced by the GC in that run.* The back-calculation algorithm starts by assuming the ideal temperature and t_M vs. T profiles and uses them to project the retention times of all the n -alkanes. Then, in each of the subsequent iterations, it makes a small adjustment to the shape of either the temperature or the t_M vs. T profile and projects the retention times of the n -alkanes again using the adjusted profiles. If the change to the profiles improves the accuracy of the retention projections, the change is kept, otherwise it is rejected. The optimization continues until the differences between the measured and projected retention times of the n -alkanes are minimized. Figure 1 shows an example of back-calculated temperature and t_M vs. T profiles back-calculated from three different GC-MS systems, each running nominally the same method. There are small, but important differences between them that would otherwise be difficult to measure.

In this manuscript, we discuss a new protocol that uses the back-calculation methodology to account for non-idealities in each of six temperature programs that are used to measure k vs. T relationships (specifically, to measure the $H(T_0)$, $S(T_0)$, and C_p parameters). First, the accuracy of the k vs. T relationships measured from the temperature programs are compared to the k vs. T relationships measured via a series of isothermal measurements. Then, to test how accurate retention projections are using the new k vs. T relationships, we used them to project retention times in 25 runs collected among 5 other labs. The distribution of error in those retention projections is compared to the distribution of error when isothermally-measured k vs. T relationships are used to project the retention times. Finally, a user-friendly software implementation of the methodology is described.

2. Experimental

2.1 Test mixture

The test mixture contained a set of 25 n -alkanes (C7–C26, C28, C30, C32, C34, and C36) along with 12 test compounds. The test compounds were chosen to represent all five types of interactions common in GC (as represented by the Abraham descriptors) [23–27]. There are hydrogen bond donors (e.g. phenol, resorcinol, and 1-naphthol), hydrogen bond acceptors (e.g. N,N -dimethylisobutyramide, benzamide, and dextromethorphan), compounds that interact by π and/or lone pair interactions (e.g. ethylbenzene, naphthalene, and anthracene), and compounds that interact by dipole-dipole and dipole-induced dipole interactions (e.g. N,N -diethylacetamide, 4-nitroaniline, and caffeine), and all test compounds vary widely in their gas-liquid partition coefficients [28,29]. All standard compounds were

dissolved in ethyl acetate at 100 μ M concentration. All chemicals and solvents were purchased from Sigma-Aldrich® (St. Louis, MO), Alfa Aesar® (Ward Hill, MA), or TCI America (Portland, OR).

2.2 Instrumentation

Two GC-MS instruments were used, which we call GC #1 and GC #2. Both GC instruments were Hewlett Packard (HP, Palo Alto, CA) Model 5890 equipped with an HP 5970 single quadrupole mass spectrometer. We used He carrier gas (99.999% pure), deactivated, straight quartz liners (2 mm inner diameter) containing deactivated quartz wool, an inlet temperature of 290 °C, and a transfer line temperature of 320 °C. GC #1 was used to measure k vs. T relationships for the n -alkanes and the 12 test compounds from isothermal runs (see section 2.3). Both GC #1 and GC #2 were used to measure k vs. T relationships for the 12 test compounds from the six temperature programs (see Results and Discussion). DB-5MS UI columns (30 m long, 0.25 mm inner diameter, 0.25 μ m film thickness) were used on both instruments.

2.3 Measurement of k vs. T relationships from isothermal runs

A detailed description of the isothermal measurements is available elsewhere [22] along with the measurements themselves. In short, we measured isothermal retention factors for each of the 37 compounds in the test mixture at 20 °C intervals from 60 °C to 320 °C, using N₂ as the t_M marker. All retention times were measured from the apex of each peak. The measurements were made on GC #1 using He carrier gas and a 30:1 split.

2.4 Temperature measurements

The GC oven temperature was measured using a custom, immersible secondary platinum resistance temperature probe with an accuracy of ± 0.05 °C (Burns Engineering, Minnetonka, MN) couple to an Agilent Technologies 34410A digital multimeter. The entire probe was placed in the oven, held off the walls and slightly above the GC column by wire supports. At least 15 minutes were allowed for the temperature to reach a steady state.

2.5 Software

GC Retention Database Builder was compiled for compliance with the Java 1.7 (Oracle, Redwood Shores, CA) runtime environment. It includes the Unidata netCDF library version 4.2 (Unidata®, Boulder, CO), the Savitzky-Golay filter library version 1.2 by Marcin Rze nicki (<http://code.google.com/p/savitzky-golay-filter/>), the jmzML library [30], and the jmzReader library [31]. The source code may be downloaded from <http://www.retentionprediction.org/gc/development>.

3. Results/Discussion

3.1 Determination of k vs. T relationships from temperature programmed runs

For all experiments, a test mixture containing 25 n -alkanes and 12 chemically diverse test compounds was used. The 25 n -alkanes were used to back-calculate the temperature profiles and t_M vs. T profiles (using k vs. T relationships for each n -alkane that were measured from

isothermal runs [22]), and the 12 test compounds were used to assess the accuracy of k vs. T relationships measured from the set of temperature-programmed runs.

A minimum of three temperature programs were necessary to determine $H(T_0)$, $S(T_0)$, and C_p for each test compound, but we chose to use six temperature programs to improve the accuracy of the measured k vs. T relationships (i.e., the accuracy of $H(T_0)$, $S(T_0)$, and C_p) and to provide some redundancy in case a compound elutes in the solvent delay in some of the temperature programs. Table 1 shows the six temperature programs we selected to measure the k vs. T relationships of the test compounds.

These six temperature programs were designed to measure k vs. T relationships for compounds encompassing a wide range in retention. Program A probes the region of k vs. T relationships with larger retention factors. This program begins with a 5 min isothermal hold at a low temperature (60 °C) followed by the shallowest ramp (4.3 °C/min) of the six programs, followed by another isothermal hold at the coolest temperature (270 °C) that still elutes the largest n -alkane, n -hexatriacontane, in a reasonable amount of time. On the other hand, program F probes the region of k vs. T relationships with smaller retention factors. It begins with a much higher initial temperature (160 °C) followed by a steep ramp (26 °C/min) and finishes with an isothermal hold at 320 °C. The total time required to run all six temperature programs was approximately 6 hours. This includes the time required for temperature equilibration before each run.

Figure 2 shows a flow chart describing the general approach used in this work to calculate a test compound's $H(T_0)$, $S(T_0)$, and C_p from the six temperature programs. First, a sample containing the test compound is spiked with the 25 n -alkanes and run under each of the six temperature programs. Then the retention times of the n -alkanes are entered into an algorithm which uses them to back-calculate the effective temperature and t_M vs. T profiles produced by the GC system in each run. Finally, the retention time of the test compound in each run, combined with the back-calculated temperature and t_M vs. T profiles in each run, are used to solve for its

$H(T_0)$, $S(T_0)$, and C_p . For this last step, we used the Levenberg-Marquardt fitting algorithm [32,33]. The algorithm searched for the values of $H(T_0)$, $S(T_0)$, and C_p that minimized the square of the difference between the projected and measured retention times in each of the six temperature programs. We found that this algorithm often times converged on a local instead of the global minimum, so the algorithm was always repeated 100 times, each time with different, randomly selected initial values of $H(T_0)$, $S(T_0)$, and C_p . The solution that gave the best fit was chosen. This approach proved to be reliable—the optimal solution was usually found within the first five fits. Using a Java implementation of the algorithm, all 100 fits can be completed in under a minute.

3.2 Accuracy of k vs. T relationships measured from temperature programs

Table 2 compares retention factors measured for just one of the 12 test compounds, N,N -dimethylisobutyramide. The isothermally measured retention factors ($k_{\text{isothermal}}$) are shown along with retention factors calculated for the same temperatures (k_{program}), using the k vs. T relationships that were determined from the six temperature programs. Isothermally

measured retention factors ($k_{\text{isothermal}}$) are the standard with which we compare because they are measured directly and therefore subject to less error. The k vs. T relationships were measured on two different GC-MS instruments. The first instrument, GC #1, is the same instrument that was used to collect the isothermal retention factors two years prior. The second instrument, GC #2, is of the same make/model as GC #1. We selected an instrument of the same make/model to emphasize the importance of instrument-related sources of bias. Even though the two GC's are of the same make/model, it will be shown that the relatively small differences between them are enough to significantly bias the k vs. T data measured from them.

On each GC, system non-idealities were taken into account to three different levels when determining k vs. T relationships from the six temperature programs. The first and simplest level, labeled “No back-calculation”, made no attempt to account for any system non-idealities. It assumed that the temperature program and the t_M vs. T profiles were perfectly ideal. This assumption led to major differences between the values of $k_{\text{isothermal}}$ and k_{program} on each of the two GC systems, with an overall error in k_{program} of $\pm 27\%$ on one GC and $\pm 12\%$ on the other. Figure 3 shows the k vs. T relationships (dashed lines) as well as the isothermally measured retention factors.

The second level, labeled “ t_M profile back-calculated”, still assumed the ideal temperature programs, but the t_M vs. T profiles from each of the six temperature programs were back-calculated. This version represents a best case scenario of the approach used in the report described by McGinitie et al. [18] where the column length and inner diameter were measured to account for differences in t_M . It is “best case” because when the t_M vs. T relationship is back-calculated, it not only takes into account non-idealities in the column length and inner diameter, but also the inlet pressure or flow rate. It can also accommodate temperature calibration error to a small degree by changes to the t_M vs. T profile, though it cannot *fully* accommodate it because it is not properly accounting for it. Even so, the k vs. T relationship measured from GC #2 still shows a strong bias, yielding values of k_{program} that are $\pm 5.8\%$ in error. On the other hand, the k vs. T relationships measured from GC #1 more closely match the relationship measured isothermally, being only $\pm 0.78\%$ in error. This is probably because the temperature calibration was slightly different between GC #1 and GC #2, and the isothermal retention factors were all measured on GC #1. Using a temperature probe accurate to ± 0.05 °C, we found that this was indeed the case, with GC #1 averaging about 3 °C warmer than GC #2 (see Figure 4).

In the third level, both the temperature and t_M vs. T profiles were back-calculated for each of the six temperature-programmed runs (labeled “ T and t_M profiles back-calculated”). This approach gave the most accurate k vs. T relationships on both GC systems: 0.56% error on GC #1 and 0.41% error on GC #2. This shows that temperature non-idealities can strongly bias the measured k vs. T relationships. After accounting for temperature non-idealities on GC #2 by back-calculating the temperature profiles in each of the six runs, the accuracy of the k vs. T relationship improved 14-fold. The accuracy on GC #1 improved as well, possibly because the temperature calibration drifted slightly over the two years since the isothermal retention factors were measured. The fact that the two GC systems yielded k vs. T relationships with virtually the same amount of error also suggests that the majority of

system non-idealities were taken into account and other, instrument-independent sources of error then dominated.

Table 3 shows the $H(T_0)$, $S(T_0)$, and C_p values measured for all 12 of the test compounds and the accuracy of the resulting k vs. T relationships when compared with the isothermal retention factors. The same trends are present as were seen for *N,N*-dimethylisobutyramide. Overall, the accuracy of the k vs. T relationships when both temperature and hold-up time profiles were back-calculated was $\pm 0.75\%$ on GC #1 and $\pm 0.95\%$ on GC #2. For comparison, when the isothermally-measured retention factors for each of the 12 test compounds are fit to eq. 3, the fit is only accurate to $\pm 0.46\%$. Therefore, the k vs. T relationships could not possibly be more accurate than $\pm 0.46\%$. Those measured using the new methodology show less than 2-fold more error than this lower limit.

It is important to note that even though the error in the k vs. T relationships is small, uncertainty in the $H(T_0)$, $S(T_0)$, and C_p values is relatively large. Different values of $H(T_0)$, $S(T_0)$, and C_p can be selected that give nearly identical k vs. T relationships. When the values measured from GC #1 (with both temperature and t_M vs. T profiles back-calculated) are compared to those measured on GC #2, values of $H(T_0)$ for a given compound vary by 3.7%, $S(T_0)$ varies by 4.3%, and C_p varies by 33%.

As an aside, it may be puzzling at first glance that the k vs. T relationships measured on GC #2 match the isothermally-measured retention factors measured on GC #1 so closely even though the isothermally-measured retention factors are biased by temperature calibration error of GC #1. The reason it did not matter is because the temperature profile from GC #2 was back-calculated using k vs. T relationships of the *n*-alkanes that were measured isothermally on GC #1. By using those k vs. T relationships to back-calculate temperature profiles, the temperature calibration of GC #1 (as it was two years ago) effectively became the standard to which all other GCs were “calibrated”. In the future, we plan to re-measure the k vs. T profiles of the *n*-alkanes using an accurate temperature probe so that back-calculated temperature profiles are accurate relative to the true temperature.

3.3 Use of the measured k vs. T relationships to project retention on other GC-MS systems

To assess the accuracy of the new k vs. T relationships in a different way, we tested the accuracy of retention times projected from them in five other laboratories. Each lab was asked to run the test mixture under five different methods and the retention times of the 12 test compounds were projected in each one (details of the experiments are given in [7]). Previously, we used the isothermally-measured k vs. T relationships measured on GC #1 to project the retention times. It was found that the accuracy of the retention projections (when normalized to the theoretically expected amount of error for a given method, σ_{expected}) was independent of the lab that ran the sample or the method under which the sample was run. Figure 5a shows a histogram of the error in the retention projections from each method in each lab (282 total retention projections), which closely followed a normal distribution. The width of this distribution is important as it is used by the retention projection software to calculate the appropriate retention time tolerance windows to use with each projected retention time [7]. We wondered if a similar distribution of error would be obtained when the new k vs. T relationships measured on GC #2 were used to project the retention times of

the test compounds in those same runs. If the new k vs. T relationships were significantly biased, the distribution of error in the retention projections would be wider. That would then indicate that the retention time tolerance windows would need to be relaxed when using k vs. T relationships measured by the new approach on different GC systems.

Figure 5b shows a histogram of the normalized error in the retention times that were projected using the k vs. T relationships measured on GC #2 using the new methodology. The distribution shifted to slightly more negative values and became a bit taller in the center, but overall, the distribution is very similar, if not slightly narrower. Thus, even though the k vs. T relationships were measured on a different GC system, and even though they were measured from temperature programs instead of isothermal runs, retention times were projected from those k vs. T relationships with nearly the same accuracy as before. This supports the premise that the methodology enables measurement of k vs. T relationships from temperature programmed runs with very little bias, regardless of the GC system used to measure them. This also potentially means that the same calculations developed previously [7] to calculate retention time tolerance windows remain correct regardless of the GC system used to measure the k vs. T relationships.

3.4 GC Retention Database Builder software

In order to make the new methodology accessible and easy to use, we developed free software that walks a user through the process of measuring k vs. T relationships. Before using the software, a user will have spiked a sample (containing the new compounds for which they wish to measure k vs. T relationships) with the test mixture (containing both the n -alkanes and the 12 test compounds) and they will have run the sample under the six temperature programs. Then they load the GC Database Builder application from www.retentionprediction.org/gc/database. Figure 6 shows the workflow of the software. There are seven tabs; the first six correspond to each of the six temperature programs. In the top panel of Figure 5, the tab for Program C is open. There, the user first enters the experimental retention times they measured for each of the n -alkanes. (The software is also able to extract the retention times from a GC-MS data file in either mzML, mzXML, or CDF formats.) In the next step (second panel), the retention times of the n -alkanes are used to back-calculate the temperature and t_M vs. T profiles produced by the GC in that run. In the next step (third panel), the back-calculated profiles are used to project the retention times of the 12 test compounds and they are compared to the measured retention times of the 12 test compounds. Based on the accuracy of the retention projections, the system is given a rating that describes its suitability (the system suitability check is described in more detail elsewhere [7]). Anything in the green or yellow region is considered a “pass”, and anything in the red region is considered a “fail”. This step is necessary to avoid bias from experimental factors affecting retention that are not properly taken into account by the retention projection methodology such as a dirty liner or column. In those cases, the system suitability check will fail until the problem is fixed. These steps are repeated 5 more times for the other 5 temperature programs.

In the final step (bottom panel), the user then enters the retention times they measured for a new compound in each of the six temperature programs, along with optional information

describing the compound (e.g. its name, formula, etc.). Lastly, the $H(T_0)$, $S(T_0)$, and C_p parameters of the new compound are solved by 100 iterations of the Levenberg-Marquardt algorithm as described earlier. The results of the six system suitability checks are also shown along the left-hand side of the window. We set a tentative threshold that at least 5 of the 6 system suitability checks must pass in order for the data to be admissible into the online database we are developing.

4. Conclusions

The new methodology described here potentially overcomes the major challenges associated with the development of a shared, unbiased k vs. T database for gas chromatography. The approach is relatively easy and fast, using a set of six temperature programs to capture the k vs. T relationships, and it requires no extra equipment or time-consuming measurements of GC system properties (e.g. unwinding the column to measure its length). Past approaches using temperature programs to measure k vs. T relationships were found to introduce major bias due to non-idealities in the GC system. However, by using the retention times of a series of n -alkanes to back-calculate the effective temperature and t_M vs. T profiles produced by the GC in each of the six temperature programmed runs, the vast majority of the non-idealities were taken into account. When only the t_M vs. T profile was back-calculated, error in the k vs. T profiles dropped considerably, but when both the t_M vs. T and temperature profiles were back-calculated, error in the k vs. T relationships dropped to less than 1%, 12 to 32-fold less than when the ideal profiles were assumed. It is therefore important to account for *both* types of non-idealities—even though the two GC systems used in this work were of the same make/model, there were significant differences between them that would otherwise bias k vs. T relationships measured from them. When both the temperature and t_M vs. T non-idealities were taken into account, the measured k vs. T relationships were nearly as accurate as the same relationships measured isothermally, being at most 2-fold less accurate. For many purposes, the speed and simplicity of the new approach may outweigh the relatively small loss in accuracy.

Despite the apparent loss in accuracy, retention projections calculated from them had the same distribution of error as when the isothermally-measured k vs. T relationships were used to project retention in 25 runs collected by five other labs. Therefore, retention time tolerance window calculations likely remain valid even when the k vs. T relationships are measured with the new methodology, and even when they are measured with different makes/models of GC systems.

Software was developed to make the methodology easy to use. It automatically extracts the retention times of the n -alkanes and the test compounds from each of the six temperature programmed runs, back-calculates the effective temperature and hold-up time profiles for each one, checks the system suitability, and then uses the retention times of a new compound in each run to calculate its $H(T_0)$, $S(T_0)$, and C_p parameters. In the near future, we plan to build a large database of k vs. T relationships using this approach. The online software makes it possible for others to begin building such databases of their own and/or to contribute to the one available at www.retentionprediction.org/gc.

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Highlights

- ☐ Retention factor vs. T relationships can be measured from six temperature programs
- ☐ Measured this way, they are strongly biased by GC system non-idealities
- ☐ The retention times of standards may be used to back-calculate the non-idealities
- ☐ Once they are taken into account, the measured k vs. T relationships are unbiased
- ☐ Software was developed to make the new methodology accessible and practical

Figure 1.

Example of temperature profiles (top) and t_M vs. T profiles (bottom) back-calculated from three different GC-MS instruments, all running nominally the same method compared with the ideal profiles (dashed lines). The differences are most pronounced in the t_M vs. T profiles, but there are also significant differences in the temperature profiles.

Figure 2.

Flow chart describing the process used in this work to determine $H(T_0)$, $S(T_0)$, and C_p (i.e., the k vs. T relationships) of a test compound from six temperature programmed runs. A sample is spiked with a set of n -alkanes and run in the six temperature programs A–F. The retention times of the n -alkanes are then used to back-calculate the effective behavior of the GC (T vs. time and t_M vs. T profiles) in each run. Then a test compound's $H(T_0)$, $S(T_0)$, and C_p parameters are solved numerically, taking into account its retention times in each run and the effective behavior of the GC in each run.

Figure 3.

Comparison of the isothermally measured retention factors for *N,N*-dimethylisobutyramide (black dots) with the k vs. T relationships measured from the six temperature programs on GC #1 (blue lines) and GC #2 (red lines). Dashed lines show the k vs. T relationships calculated with neither profile back-calculated, dotted lines show k vs. T relationships determined with back-calculation of t_M vs. T profiles, and the solid lines show k vs. T relationships determined with back-calculation of both temperature and t_M vs. T profiles.

Figure 4.

Temperature calibration error as a function of the set temperature for the two GC instruments used to measure k vs. T relationships.

Figure 5. Normalized error in the retention projections for each of the 12 test compounds in five different methods across five different labs **a)** using the isothermally measured k vs. T relationships and **b)** using the k vs. T relationships measured from the six temperature programs. The red line shows the normal distribution that best fits the histogram in panel **a**. The same distribution is shown overlaid on histogram **b**. The normalized error is given as $(t_{R,meas} - t_{R,proj}) / \sigma_{expected}$, where $t_{R,meas}$ is the measured retention time and $t_{R,proj}$ is the

projected retention time, and σ_{expected} is the expected error for a specific retention projection in a specific method (see [7] for details).

Table 1

The Six Temperature Programs Used to Determine $H(T_0)$, $S(T_0)$, and C_p

Program	Initial T	Initial Hold Time	Ramp Rate	Final T	Final Hold Time	Total Time Required
A	60 °C	5 min	4.3 °C/min	270 °C	50 min	104 min
B	80 °C	5 min	8.7 °C/min	280 °C	40 min	68 min
C	100 °C	5 min	13.0 °C/min	290 °C	30 min	50 min
D	120 °C	5 min	17.3 °C/min	300 °C	20 min	35 min
E	140 °C	5 min	21.7 °C/min	310 °C	20 min	33 min
F	160 °C	5 min	26.0 °C/min	320 °C	15 min	26 min

Table 2

Comparison of Isothermal Retention Factors for the Test Compound *N,N*-dimethylisobutyramide Measured Isothermally ($k_{\text{isothermal}}$) and Measured from Six Temperature Programs (k_{program})

<i>T</i> (°C)	$k_{\text{isothermal}}$	k_{program} from GC #1 ^a		k_{program} from GC #2 ^a	
		<i>T</i> and <i>t_M</i> profiles back-calculated	<i>t_M</i> profile back-calculated	<i>T</i> and <i>t_M</i> profiles back-calculated	<i>t_M</i> profile back-calculated
		No back-calculation		No back-calculation	
60	5.434	5.386	5.385	6.423	5.400
					5.442
					5.652
80	2.276	2.268	2.265	2.729	2.273
					2.272
					2.266
100	1.077	1.073	1.078	1.360	1.079
					1.031
					0.974
120	0.563	0.560	0.569	0.774	0.566
					0.503
					0.445
140	0.318	0.318	0.328	0.491	0.323
					0.260
					0.214
160	0.193	0.193	0.204	0.342	0.198
					0.142
					0.108
180	0.124	0.125	0.135	0.257	0.129
					0.081
					0.057
200	0.084	0.085	0.094	0.207	0.089
					0.049
					0.031
Relative Error:^b		0.56%	0.78%	27%	0.41%
					5.8%
					12%

^a k_{program} is the retention factor determined for the specified temperature using the $H(T)$, $S(T)$, and C_p values calculated for *N,N*-dimethylisobutyramide using the six temperature programs.

^b Error is given as the relative root mean square error (RMS) between $k_{\text{isothermal}}$ and k_{program} for values of $k_{\text{isothermal}}$ that were greater than 0.5.

Table 3
 $H(T_0)$, $S(T_0)$, and C_p Values Determined for Each Test Compound from Six Temperature Programs on Two Different GC Instruments (with Different Levels of Back-Calculation) and Error in the Retention Factors Calculated from them Relative to those Measured Isothermally

GC #1	T and t_M profiles back-calculated				t_M profiles back-calculated				No back-calculation			
	$H(T_0)$	$S(T_0)$	C_p	Error in k_{program}^a	$H(T_0)$	$S(T_0)$	C_p	Error in k_{program}^a	$H(T_0)$	$S(T_0)$	C_p	Error in k_{program}^a
ethylbenzene	-39444.9	-112.581	45.22	0.49%	-41017.3	-117.817	72.9717	0.98%	-46197.9	-133.541	174.878	31%
naphthalene	-50104.5	-124.031	54.1712	1.1%	-51693.5	-128.871	67.73	0.38%	-57326.4	-145.368	128.865	21%
anthracene	-71439.4	-154.218	82.1464	0.93%	-67645.1	-143.649	63.3202	1.3%	-52041	-99.8546	-7.80619	22%
<i>N,N</i> -diethylacetamide	-48607.7	-131.654	77.0616	0.33%	-49824.9	-135.611	93.3733	0.46%	-56112.4	-154.767	186.746	25%
4-nitroaniline	-72284.6	-165.585	108.298	1.1%	-71746.6	-164.052	105.486	1.1%	-87849.7	-210.126	215.465	23%
caffeine	-78153.5	-169.398	95.2559	0.85%	-73623.4	-156.807	72.6252	1.5%	-56753.7	-110.214	0.73252	23%
phenol	-49817.1	-136.731	87.3468	0.29%	-51322.4	-141.635	107.86	0.73%	-57993.7	-162.096	210.436	27%
resorcinol	-68494	-172.304	132.748	0.51%	-69766.5	-176.2	143.475	0.55%	-79482.9	-204.94	233.86	20%
1-naphthol	-69417.2	-161.997	105.737	0.94%	-68267.8	-158.475	96.3585	0.86%	-92530.8	-228.987	267.777	21%
<i>N,N</i> -dimethylisobutyramide	-46946.9	-128.147	66.4981	0.56%	-48380.3	-132.811	86.0416	0.78%	-54879	-152.706	186.359	27%
benzamide	-62287.3	-151.382	93.8409	0.55%	-63110.7	-153.822	99.7071	0.57%	-69898.2	-173.356	159.966	20%
dextromethorphan	-90770	-189.223	114.78	0.67%	-107431	-232.871	181.19	3.2%	-73506.1	-143.914	54.7685	23%
Overall error in k_{program}^a:												
GC #2	T and t_M profiles back-calculated				t_M profiles back-calculated				No back-calculation			
	$H(T_0)$	$S(T_0)$	C_p	Error in k_{program}^a	$H(T_0)$	$S(T_0)$	C_p	Error in k_{program}^a	$H(T_0)$	$S(T_0)$	C_p	Error in k_{program}^a
ethylbenzene	-43427	-125.478	94.9818	1.2%	-33346.2	-92.224	-72.708	8.8%	-23335.4	-58.9036	-237.196	13%
naphthalene	-49371.5	-121.83	49.3824	1.5%	-42637	-100.785	-17.201	4.4%	-43304.8	-101.373	-41.771	10%

anthracene	-72263.1	-156.608	87.3599	1.2%	-72609.1	-157.635	88.9527	1.7%	-70520.3	-150.239	64.9209	5.2%
N,N-diethylacetamide	-48708.6	-131.976	79.3697	0.27%	-40985.3	-106.763	-28.490	4.6%	-38895.8	-99.102	-85.0468	10%
4-nitroaniline	-68396.3	-154.362	84.594	1.1%	-65536.7	-145.956	65.0033	1.7%	-83007.1	-195.068	159.129	4.5%
caffeine	-80668.1	-176.484	109.215	1.1%	-81823.9	-179.701	114.614	1.2%	-69274.1	-143.103	36.1407	5.8%
phenol	-49153.1	-134.638	83.1189	1.3%	-45915.2	-123.657	22.1814	4.5%	-42781.4	-112.687	-46.6067	11%
resorcinol	-69020.5	-173.915	137.917	0.46%	-54889.2	-130.673	17.7447	3.2%	-62336.3	-151.305	44.0145	9.6%
1-naphthol	-67438.2	-156.222	93.0811	0.82%	-59557.4	-132.949	38.5366	1.8%	-96045.1	-239.443	293.034	22%
N,N-dimethylisobutyramide	-47487.3	-129.885	73.9911	0.41%	-40737.8	-107.673	-28.278	5.8%	-39367	-102.352	-76.5111	12%
benzamide	-62158.4	-151.021	93.7905	0.30%	-53570.7	-125.007	24.971	2.4%	-58470.6	-137.736	30.8372	11%
dextromethorphan	-88906.3	-184.454	107.559	0.87%	-84484.7	-172.905	90.0995	1.6%	-83955.1	-169.361	72.6844	4.5%
Overall error in k_{program}:				0.95%				4.1%				11%

^a Error is given as the relative root mean square error (RMS) between $k_{\text{isothermal}}$ and k_{program} over all measured temperatures for which values of $k_{\text{isothermal}}$ were greater than 0.5.